Abstract

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Project Title: Screening for Compounds That Modulate Insulin Promoter Activity in TRM-67 Cells

Abstract: DESCRIPTION (provided by applicant): The assay being proposed here will screen for small molecule compounds that modulate insulin promoter activity. The assay is based upon a human pancreatic endocrine cell line, TRM-6, that was derived from fetal islets. To develop these cells as the basis for a high throughput assay, they were engineered to express an insulin promoter-fluorescent reporter protein transgene using a lentiviral vector. In addition, the cell line has been engineered to express a panel of transcription factors that together stimulate insulin gene activity; thus, the cells express substantial levels of endogenous insulin mRNA but less than produced by a healthy pancreatic ?-cell. Thus, although broadly designed to detect compounds that affect Insulin gene expression, either positively or negatively, the screen is specifically engineered to increase the likelihood of detecting compounds that complement the activities of the known transcription factor inputs controlling insulin production. The secondary assay used to verify hits will be evaluation of the biological effect on the endogenous insulin gene. Preliminary Studies indicate that the insulin promoter-fluorescent reporter transgene mimics the activity of the endogenous insulin gene. Optimization of the assay characteristics, including the cell seeding density and tamoxifen concentration yielded a z' of 0.6. A small scale screen of 8,000 compounds from the ChemBridge DiverSet library identified multiple compounds that induced and repressed the insulin promoter-GFP transgene. A primary confirmatory assay yielded a true positive rate of approximately 50% of those scored initially as hits. Using the secondary assay of quantitative insulin RT-PCR found that these confirmed hits had significant effects on endogenous insulin promoter activity. Discovering compounds that modulate the insulin promoter has the potential to identify signaling pathways that are involved in the establishment and maintenance of mature ?-cells.

Thesaurus Terms:
High throughput screening, assay, insulin promoter activity, TRM-67 Cells, TRM-6, promoter-fluorescent reporter protein transgene, lentiviral vector, endogenous insulin mRNA, tamoxifen, ChemBridge DiverSet library, quantitative insulin RT-PCR

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